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DEVELOPMENT OF THE IMMATURE STAGES OF ANASTREPHA SERPENTINA IN RELATION TO TEMPERATURE[1]

By JOHN G. SHAW, *assistant entomologist*, and DONALD F. STARR, *chemist, Division of Fruitfly Investigations, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

During the past several years large numbers of adults of *Anastrepha serpentina* (Wied.) have been found in the citrus groves of the lower Rio Grande Valley of Texas and Tamaulipas, Mexico. In 1934 James Zetek found sweet oranges purchased from the Panama City market to be infested with *A. serpentina*. In 1939 larvae were found in grapefruit at Mission, Tex., by employees of the Bureau of Entomology and Plant Quarantine. Hosts of economic importance in Mexico are chicozapote (*Achras zapota* L.), zapote mamey (*Calocarpum mammosum* (L.) Pierre), and peach (*Prunus persica* (L.) Stokes). The southern limit of *A. serpentina* given by Stone [2] is São Paulo in Brazil. In view of the threat of this insect to the citrus fruit industry a study of its development was undertaken at the Mexico City laboratory.

EGG STAGE

METHODS USED IN STUDY

Populations of fruitflies were reared from infested fruits of chicozapote and zapote mamey, but for the studies on the development of the species chicozapote and peach were used.

The flies to be used for supplying eggs were confined in glass-and-muslin cages, close to 12 inches in each dimension, and held in a large cabinet at 25° C. and approximately 60 percent relative humidity. Under these conditions the flies deposited the first eggs 16 days after emerging. The first mating was not observed until the twentieth day. Mating generally took place between 5 and 6 p. m. and lasted from 25 to 57 minutes.

The technique used for obtaining fruitfly eggs was that developed in Florida in 1929 during research on the Mediterranean fruitfly, *Ceratitis capitata* (Wied.) (unpublished). An outer section of chicozapote or peach was cut with the skin adhering to it, and most of the flesh was removed to leave a thin-walled shell (fruit skin), which the ovipositor of the fly could pierce. Each arc of fruit skin was fastened with paraffin to a 2½- by 2¾-inch glass plate. Since the eggs usually adhered to the inner side of the fruit skin, the laborious task of looking

[1] Received for publication August 24, 1944.

[2] STONE, A. THE FRUITFLIES OF THE GENUS ANASTREPHA. U. S. Dept. Agr. Misc. Pub. 439, 112 pp., illus. 1942.

for eggs in the flesh of whole fruits was thus avoided. The eggs, usually in groups of 4 to 12, could be easily observed through the glass. The fruit skin was removed from the glass when it became necessary to transfer the eggs.

The mounted fruit skins were placed in stock cages of flies for 2 or 3 hours between 1 and 7 p. m., the time of day when most oviposition by *Anastrepha serpentina* takes place.[3] Eggs were removed from fruit skins by means of a camel's-hair brush and placed in rows on ink-blackened filter paper in a Syracuse watch glass. The filter paper was kept saturated with a 0.1-percent solution of cupric chloride, apparently without affecting the hatching of the eggs. Darby and Kapp [4] used this solution to control the growth of fungi when they reared "pupae" of the Mexican fruitfly (*A. ludens* (Loew)) on cotton. The watch glasses were stacked and ground to fit tightly to avoid desiccation and the escape of tiny first instars. The largest number of eggs were deposited on fruit skins placed in a row along the cage floor against the glass front nearest the source of light.

From time to time equal numbers of fruit-skin sections of ripe chicozapote and peach were placed in stock cages of flies, and the eggs deposited in each kind of fruit were counted. Preference for chicozapote was shown when 599 eggs were laid in this fruit and only 151 in peach. When the mean difference of 15 comparisons was analyzed statistically, the indicated preference was shown to be highly significant. Chicozapote might well be expected to be the preferred host because it is attacked heavily by *Anastrepha serpentina* in the field.

The eggs were incubated in six insulated cabinets, each 32 by 13 by 15 inches. Temperature was controlled by using electric lamps as a source of heat, and ice to produce low temperatures. Shallow pans of water were placed in the cabinets at 25° C. or above to maintain a relative humidity of approximately 60 percent. Temperature was controlled within $\pm 0.15°$ C. by means of toluene-mercury thermostats, and the air was circulated by 8-inch fans. A thermograph and a thermometer were kept in each cabinet. Care was taken to determine the temperature at the exact location of the biological material. At the end of the desired observation periods the containers with eggs were removed from the cabinets and quickly examined under a binocular microscope to record the hatch.

LENGTH OF INCUBATION AND HATCHING PERIODS

The incubation and hatching of eggs were studied at various temperatures between 10° and 37.8° C. The incubation period was reckoned from the time the fruit skins containing the eggs were transferred from the stock cages to the time of the last observation before the first egg hatched, and the hatching period from this observation until the last egg hatched.

When 1,000 eggs were held for 112 to 120 days at 10° C., no hatching was observed, although one-third to one-half of them appeared normal at the end of that time.

[3] Baker, A. C., Stone, W. E., Plummer, C. C., and McPhail, M. A review of studies on the Mexican fruitfly and related Mexican species. U. S. Dept. Agr. Misc. Pub. 531, 155 pp., illus. 1944.

[4] Darby, H. H., and Kapp, E. M. Observations on the thermal death points of Anastrepha ludens (Loew). U. S. Dept. Agr. Tech. Bul. 400, 19 pp., illus. 1933.

At 12.5° C. none of 167 eggs hatched during a period of 81 days. After 72 days 1 larva partially emerged, and fully developed larvae were found in several eggs upon dissection. From equation 2, which is given later, it was calculated that hatching would be expected after 63 days.

No hatch was obtained from 699 eggs held at 35° and 572 eggs held at 37.8° C. Some of the eggs held at these temperatures became enlarged and split open longitudinally, exposing partially developed larvae, but more of the eggs simply collapsed. Other eggs remained white, turgid, and apparently normal after being held for 49 days at 37.8° C.

Eggs hatched at temperatures ranging from 15° to 32.5° C. A total of 3,520 eggs were incubated and observed at several temperatures within this range. Eggs held at 15° were observed usually twice each day, and those of one group (a) held at 25° were observed every 3 hours throughout the day and night. On eggs held at 20°, 30°, and 32.5°, and on a second group (b) at 25°, the time of observations was varied in an attempt to obtain points equally spaced in terms of the logarithm of time, according to the method used by Bliss.[5] A summary of the incubation and hatching data obtained at the various temperatures is presented in table 1.

TABLE 1.—*Summary of tests on incubation and hatching of Anastrepha serpentina eggs*

Temperature (°C.)	Eggs		Incubation period	Hatching period
	Incubated	Hatched		
	Number	*Percent*	*Hours*	*Hours*
15	179	15.6	361.0	112.5
20	743	52.1	144.0	80.7
25 (a)	368	21.5	90.0	39.0
25 (b)	682	40.5	86.8	51.2
30	741	48.9	64.9	40.2
32.5	807	33.3	64.5	33.2

MATHEMATICS OF THE HATCHING PROCESS

A mathematical treatment of the hatching data was undertaken, and since satisfactory curves were fitted the essential details of the study will be described.

The asymmetric sigmoid curve obtained when the cumulative hatch was plotted against total developmental time suggested that it might be transformed to a straight line by converting the percentages of cumulative hatch to probits [6] and the time units to logarithms. The straight-line relationship was not obtained, however, until a time constant was subtracted from the total developmental time. The incubation period was used as the constant.

The introduction of the time constant into the logarithmic term to fit the probit data to a straight line is in harmony with the use of

[5] BLISS, C. I. THE CALCULATION OF THE TIME-MORTALITY CURVE. Ann. Appl. Biol. 24: 815–852, illus. 1937.

[6] BLISS, C. I. THE CALCULATION OF THE DOSAGE-MORTALITY CURVE. Ann. Appl. Biol. 22: 134–167, illus. 1935.

similar constants by other workers. Bliss [7] introduced a "threshold concentration" to straighten dosage-mortality curves, and Starr (unpublished reports) used an "approach-period equivalent" to produce straight mortality curves.

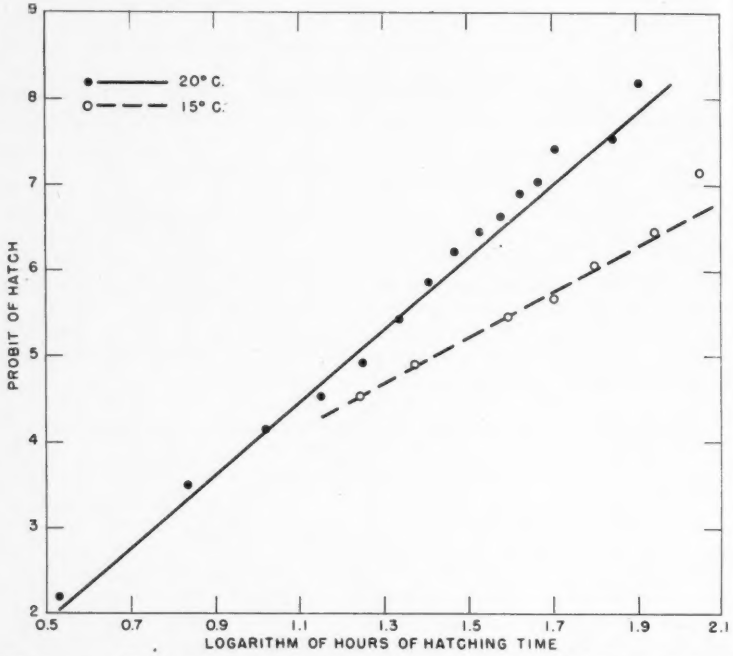

FIGURE 1.—Hatch of *Anastrepha serpentina* eggs at 15° and 20° C., in terms of probits and logarithms of $(T-I)$. At 15° $y=2.67 \log (T-361.0)+1.21$. At 20° $y=4.21 \log (T-144.0)-0.16$.

The converted hatching data for each test summarized in table 1 were plotted, and the results obtained at 15° and 20° C. are shown in figure 1. In each case approximately a straight line was indicated, which may be represented by the general equation

$$y=m\ [\log\ (T-I)]+b \qquad (1)$$

where y is the probit of the percent cumulative hatch, m is the slope of the line or the rate of hatch, T is the developmental time for the portion of the eggs represented by y, I is the incubation period, and b is a constant which corrects for the fact that the line may not pass through the origin.

The incubation period as determined in these studies is not completely independent of the number of eggs that hatch. A large sample has a broader hatching range than a small one, the first egg hatching

[7] BLISS, C. I. THE RELATION BETWEEN EXPOSURE TIME, CONCENTRATION AND TOXICITY IN EXPERIMENTS ON INSECTICIDES. Ent. Soc. Amer. Ann. 33: 721–766, illus. 1940

a little sooner and the last egg considerably later. For a given temperature the observed incubation period should be constant within about 4 percent, which is sufficiently accurate to be used as a "time constant" in these calculations. The incubation period could be determined more exactly by a combination of more frequent observations and larger numbers of viable eggs. The true value could be expected with an infinite number of viable eggs and continuous observation.

A study of the variability of the incubation period with the number of viable eggs was based on the equation for the line for 20° C. in figure 1. The incubation period of 144 hours (table 1) was found experimentally with 387 viable eggs and observations approximately every 3 hours. Assuming hourly observations and 144 hours as the true incubation period, the relation between the number of viable eggs and the observed incubation period was calculated. The expected hatch at any time for any given number of viable eggs may be calculated from the equation. When any value below 50 is selected for the percent hatch, the first egg to hatch automatically fixes the total number of viable eggs. The percent hatch as used above is not the percent of viable eggs, but the percent of the total viable eggs that have hatched at a given time. For example, at 147 hours a probit of 1.85 is calculated from the equation at 20°. The probit is equivalent to 0.082 percent. In order for the first hatched egg to be 0.082 percent hatch, there must be 1,220 viable eggs. From a group containing 1,220 viable eggs hatch should be observed at 147 hours, but not at 146; therefore, the incubation period is recorded as 146 hours in table 2.

TABLE 2.—*Expected variation in the estimation of the incubation period with the number of viable eggs of Anastrepha serpentina at 20° C.*

Number of viable eggs	Incubation period	Number of viable eggs	Incubation period
	Hours		*Hours*
20,000	145	76	148
1,220	146	33	149
233	147	18	150

If as many as 233 viable eggs were used in an experiment, and observations were taken hourly before the initial hatch, the difference in incubation period would be 2.1 percent. Even with as few as 18 viable eggs the error would be only 4.2 percent.

The two experiments which were run at 25° C. gave values of the incubation period in agreement with the theory. With 79 and 276 viable eggs the observed incubation periods were 90 and 86.8 hours, respectively. The difference was less than 4 percent.

The reaction of the first individual, a method used successfully by Baker and coworkers,[8] gives a useful measure of the incubation period, because a relatively large portion of the eggs hatch during the first part of the hatching period. The distribution becomes normal when the time units are converted to logarithms, and the reaction of the first individual is stabilized by the large percentage of the population which reacts within a short time after the first.

[8] See footnote 3, p. 266.

The effect of temperature on the hatching process may be conveniently summarized by showing the relationship of temperature to each of the constants given in equation 1. As shown in table 3, between 15° and 30° C. the incubation period (I) is inversely proportional and the rate of hatch (m) directly proportional to the temperature. The value of b is variable, but this variation is in no way correlated with the temperature. Both the incubation period and the rate of hatch indicate that 30° is close to the optimum temperature. The rate of hatch was decreased at 32.5°, whereas the incubation period was nearly the same at 30° and 32.5°.

TABLE 3.—*Observed and calculated values for incubation period and rate of hatch of Anastrepha serpentina eggs at different temperatures*

Temperature (t), ° C.	Incubation period (I)		Rate of hatch (m)		Constant (b)
	Observed	Calculated	Observed	Calculated	
	Hours	*Hours*			
15	361.0	360.5	2.67	2.89	1.21
20	144.0	142.7	4.21	3.78	−.16
25 (a)	90.0	88.9	4.43	4.66	.68
25 (b)	86.8	88.9	4.70	4.66	.73
30	64.9	64.6	5.53	5.55	−.08
32.5	64.5		4.40		1.32
Average					.62

The equations representing the effect of temperature on these two constants were obtained by the method of least squares.

$$1/I=0.0008471t-0.009933 \quad (2)$$

$$m=0.1768t+0.242 \quad (3)$$

The observed and calculated values given in table 3 indicate that the equation is fairly exact for the incubation period. The graphic presentation of the temperature effects is shown in figure 2. It will be seen that any deviation from the linear temperature-velocity relationship in the hatching process must occur above 30° and below 15° C.

By means of equations 2 and 3 it is possible to calculate the incubation period and rate of hatch at any temperature between 15° and 30° C. within the limits of error. With these calculated constants hatching curves were calculated at each of the temperatures studied. The agreement between the calculated curves and the actual observations, which is shown graphically in figure 3, is usually within 5 hours. Since no relationship was developed for the percentage of hatch as affected by the temperature, the number of viable eggs as found by experiment was taken for the calculations.

LARVAL STAGE

METHODS USED IN STUDY

Following a technique devised by W. E. Stone (unpublished), newly hatched larvae were removed from incubation dishes with a camel's-hair brush and placed individually in a notch cut into the edge of a thin slice of fruit. The slice of fruit was sandwiched between two pieces of glass (2½ by 2¾ inches) and sealed in place by dipping the edges of the glass mount into melted paraffin (fig. 4). Excess juice

was removed from the slice of fruit to avoid drowning the larva. A few holes pushed through the paraffin between the edges of the mount provided aeration. Since fruit held between glass slides disintegrated

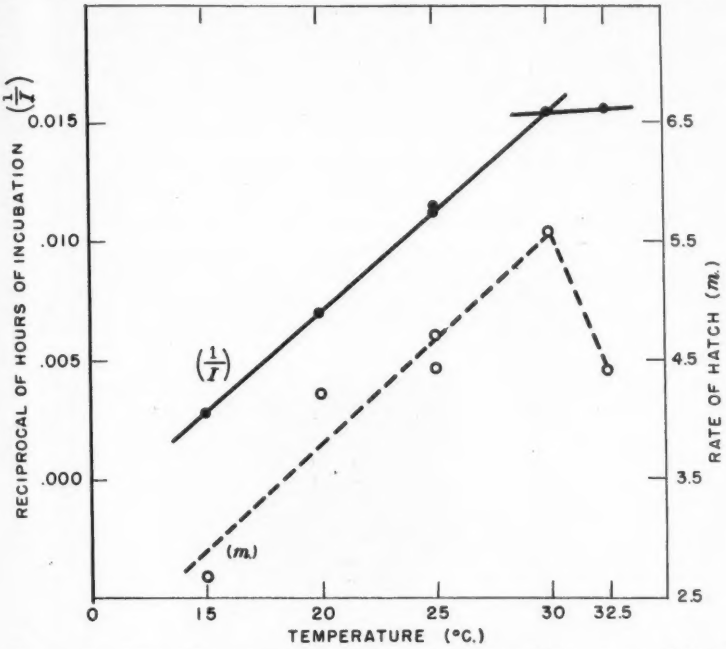

FIGURE 2.—Effect of temperature upon the incubation period and rate of hatch of *Anastrepha serpentina* eggs.

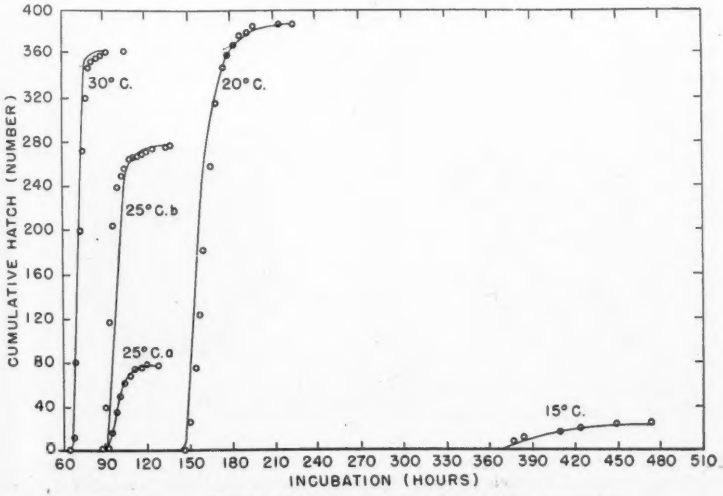

FIGURE 3.—Egg-hatching curves for *Anastrepha serpentina*.

at the higher temperatures, it was necessary to transfer larvae to fresh slices about every 24 hours. Fungus growths on fruit were reduced to a minimum through frequent changes of the media and by the use of sterilized instruments and glass plates.

The early studies on larval development were made with a white-fleshed variety of peach. Later in the season it became necessary to substitute a yellow-fleshed variety. The latter proved to be much superior, because the flesh did not darken for at least 24 hours and the cast skins and mouth hooks of larvae were readily visible against such a background.

Chicozapote when mealy ripe furnished a more satisfactory medium for rearing the larvae, but the mottled coloration of fruit cells made it difficult to locate first instars. When the fruit reached this stage, it

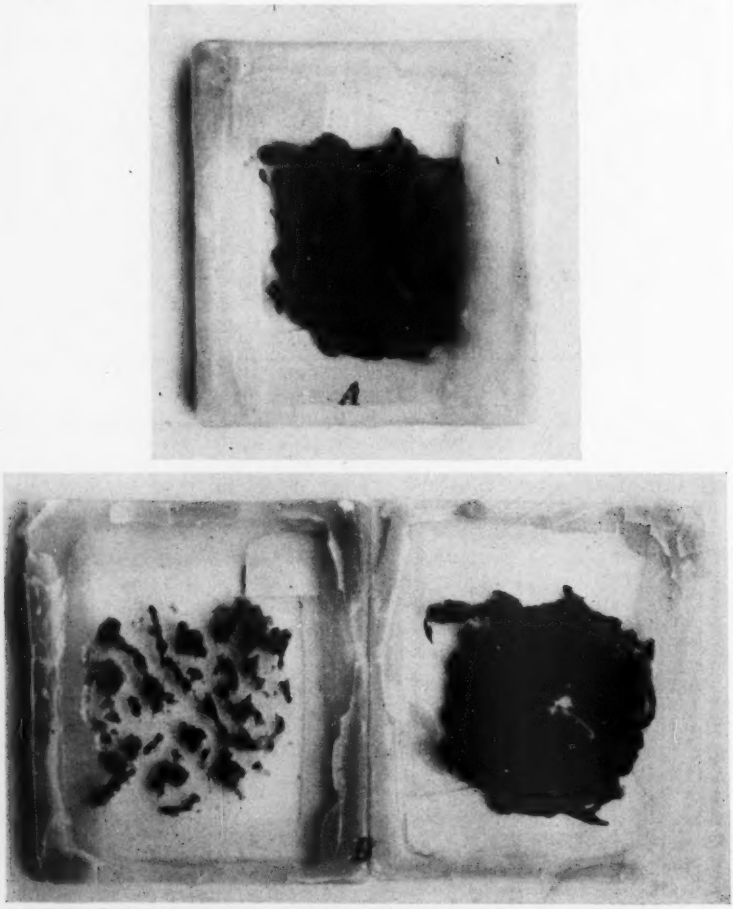

FIGURE 4.—Mount with slice of chicozapote held between glass plates, showing larva and larval tunnels of *Anastrepha serpentina*: *A*, Closed tunnels; *B*, open tunnels.

was held in a refrigerator to avoid rapid desiccation. Green fruit and fruit allowed to ripen in cold storage apparently possessed certain qualities that inhibited larval development, for in early studies at 25° C. only 1 out of 50 larvae survived when placed in green chicozapote. Later, when ripe chicozapote was used, 11 out of 15 larvae mounted were reared through the pupal stage at this temperature.

Larvae were held in a succession of sandwich mounts until late in the third stadium, when they usually became sluggish. Each larva was then placed in an excavation in a freshly cut piece of fruit, which was held over sterilized sand in a sterilized crystallization dish. The larva could thus feed on the fruit or enter the sand to pupate. Constant vigilance was required to prevent the growth of fungi. The fruit was changed often, and mycelial threads found on puparia were removed with a fine brush moistened with dilute alcohol and cupric chloride solution. The fruit was removed from the dishes as soon as the larvae pupated. Puparia were examined daily, and the sand was kept moist with a 0.1 percent cupric chloride solution.

MOLTING PROCESS

This fruitfly, in common with many other Diptera, has three larval instars. Larvae in process of molting were seen on several occasions. In one instance a first instar held at 25° C. was observed changing to a second instar. When first noticed the larva was lying on its side in a "semiparalyzed" condition and possessed mouth hooks of both instars (fig. 5). The first-instar hooks were in front and to one side of the new set of hooks, which, of course, were an integral part of the larva. The larva made vigorous efforts to free itself of the mouth hooks and skin. For about 20 minutes it kept rolling over and making frequent weak jabs with its head, occasionally contracting lengthwise and bending in such a way as to jab the end of its body. During the next 15 minutes its activity increased, the larva making numerous longitudinal propulsions posteriorly, until the cast skin was ruptured or loosened and the molting was completed. The larva then crawled away and within 4 or 5 minutes began feeding voraciously.

LENGTH OF LARVAL PERIOD

Except for 12.5° C., the larval development was studied at the same temperatures that were used in the study of the egg stage. The tests at 10° C. comprised 61 larvae that emerged from eggs incubated at 25° which were mounted in peach, and 15 larvae from eggs incubated at 20° which were mounted in chicozapote. No larvae held at this temperature attained the second instar. At 15° 1 out of 30 larvae placed in peach completed the larval stage, as compared with 4 out of 11 larvae in chicozapote.

Temperatures at 20°, 25°, and 30° C. are considered the optimum for larval survival (table 4). The length of the larval period, especially the third stadium, varied widely at 25° and 30°. Much of this variation is ascribed to differences in time spent in a semidormant condition before pupation. Some third instars that appeared to be full grown continued to be very active although they did not feed. For this reason it was not always possible to determine with certainty just when larvae should be transferred from fruit held between glass plates to fruit held over sand.

The single larva to develop at 32.5° C. spent 2.5 days in the first stadium and 1.5 days in the second. At 35° from 4 to 9 days were

spent in the first stadium and 5 to 8 days in the second, with death ensuing in the third. It appears, therefore, that the delay in development was due to the high temperature.

Of 77 larvae mounted in slices of peach at 37.8° C., none survived long enough to reach the second instar. On the other hand, the thin

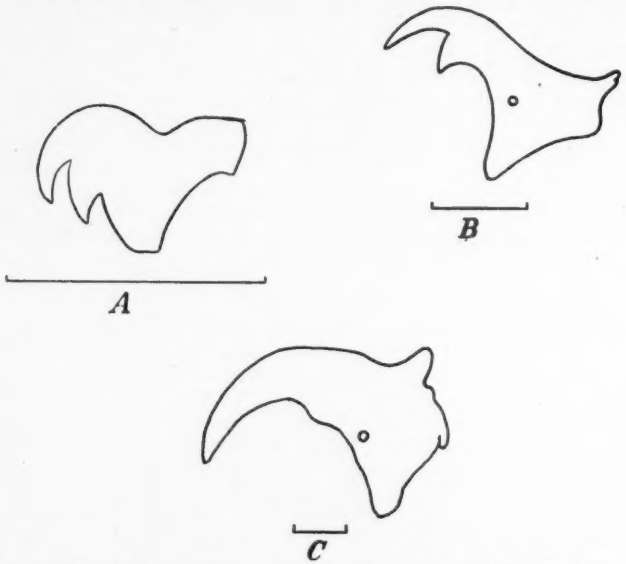

FIGURE 5.—Mouth hooks of larvae of *Anastrepha serpentina: A*, First instar *B*, second instar; *C*, third instar. The line below each figure represents 0.04 mm.

TABLE 4.—*Average length of immature stages of Anastrepha serpentina when held at various temperatures on peach and chicozapote*

Temperature (°C.)	Individuals	Egg stage	Larval period				Pupal stage	Total, egg to adult	Sex of adults [1]	
			First	Second stadium	Third stadium	Total			Male	Female
	Number	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Number*	*Number*
DEVELOPMENT ON PEACH										
15	1	[2] 16	12	17	21	50	55	121	0	1
20	2	7	7.5	4	12.5	24	26.5	57.5	1	1
25	15	4.2	3.8	3.4	9.1	19.1	16.5	39.8	6	8
30	7	3.1	3.7	3.3	10.4	17.4	12.4	33.0	2	5
DEVELOPMENT ON CHICOZAPOTE										
15	4	[2] 16	12.3	9	20.3	41.5	[3] 56.8	114.3	4	0
20	19	7	4.9	3.5	11.1	19.4	27.3	53.7	4	15
25	12	4.3	3.5	2.8	8.9	15.2	16.2	35.7	5	7
30	14	3	3.1	2.4	6.2	11.7	12.3	26.9	7	7
32.5	1	[4] 3	2.5	1.5	7	11	[5] 12	26	0	[5] 1

[1] At 25° C., only head of fly emerged from 1 puparium; sex not determined. At 30°, 3 of the 7 flies emerged with drooped wings, with somewhat malformed bodies, or with drooped wings and malformed bodies.

[2] These flies were actually incubated at 20° C.

[3] In 1940 E. W. Baker obtained the following results: From approximately 100 puparia held at 15° C., 54 flies developed in 56.4 days, and from the same number held at 32.5°, 2 flies developed in 12 days.

[4] This fly was actually incubated at 25° C.

[5] Fly was small, with drooped wings.

slices of chicozapote dried rapidly at this temperature and did not supply the larvae with suitable food. The developmental period was invariably longer when larvae were reared in peach than when reared in chicozapote.

PUPARIAL STAGE

All larvae able to complete development were reserved for observation in the puparial stage. The length of time spent in this stage is summarized in table 4. The fruit on which the larvae were reared had no effect on the length of the puparial stage. A comparison indicated that there was no difference in duration within puparia held over sand or filter paper.

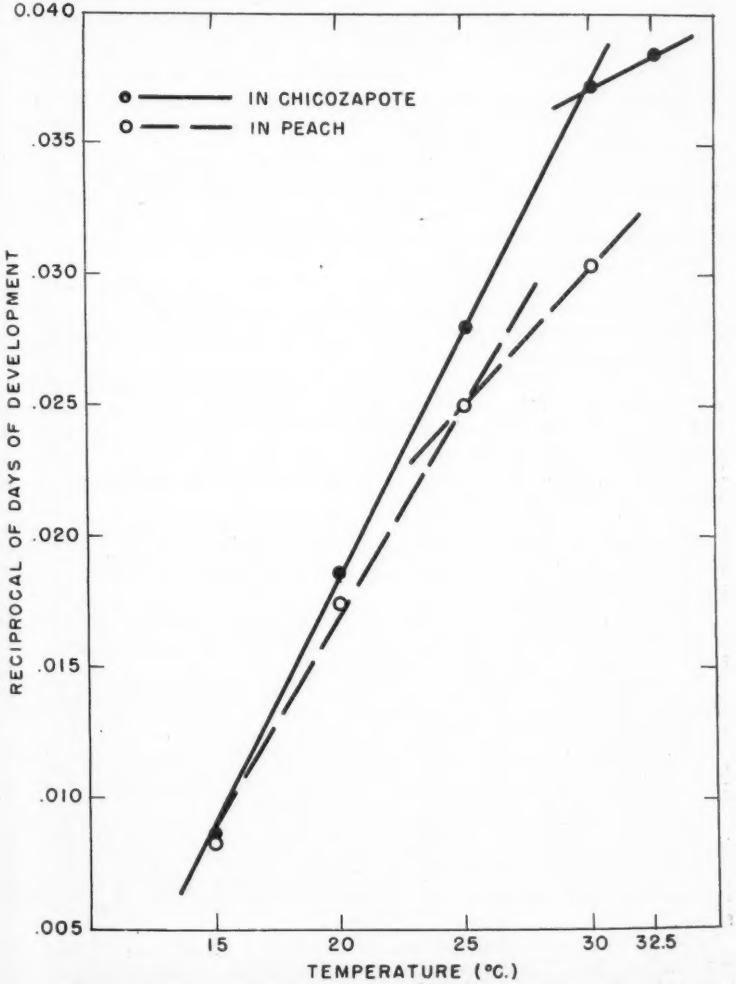

FIGURE 6.—Development of *Anastrepha serpentina* from egg to adult.

TOTAL LENGTH OF DEVELOPMENTAL PERIOD

The total length of the developmental period, egg to adult, was longer in peach than in chicozapote (table 4), and a much smaller percentage of individuals were found to complete development. When the rate of development, measured by the reciprocal of time in days, was plotted against temperature (fig. 6), linear regression lines were produced which showed the contrast in the rate of development in the two hosts. Data on the egg and larval stages indicate that the lower limit of development is between 10° and 15° C. and the upper limit between 32.5° and 35°, but somewhat lower temperatures are probably required for normal fly emergence. Flies with drooped wings, with malformed bodies, or with drooped wings and malformed bodies were observed at 30° and 32.5°. Again chicozapote appeared to be a better medium than peach.

SUMMARY

As *Anastrepha serpentina* (Wied.) is known to develop in several fruits of economic importance, the development of the immature stages of this fly was studied.

First oviposition occurred 16 days after flies began to emerge from puparia. Eggs were procured from confined flies by exposing arcs of fruit skins fastened by paraffin to glass plates. The fact that this fruitfly preferred chicozapote skins to peach skins for oviposition was shown to be highly significant.

Eggs for incubation and hatching studies were held in Syracuse watch glasses in cabinets at temperatures ranging from 10° to 37.8° C. Hatch occurred from 15° to 32.5°, and the incubation period ranged from 361 to 64.5 hours.

After the incubation period the hatching process approximated a straight line when the probit of the cumulative percent hatch was plotted against the logarithm of time. The temperature effect on the reciprocal of the incubation period and on the rate of hatch was measured so that approximate hatching curves might be calculated at any temperature from 15° to 30°.

The larvae were reared at temperatures from 15° to 32.5° C. by placing them in slices of fruit held between glass plates. Mealy-ripe chicozapote provided the better medium for larval development but peach was also used.

Molting of the first instar was observed in some detail. The second stadium was generally shorter than the first, but the third was considerably longer than either of the others.

In peach larvae developed in 50 to 17.4 days between 15° and 30° C., whereas in chicozapote development required only 41.5 to 11.7 days in the same temperature range and 11 days at 32.5°.

Holding and caring for puparia in crystallization dishes is described. The developmental time was nearly the same for puparia formed from larvae fed on peach and on chicozapote.

The total development, egg to adult, ranged from 121 days for one individual reared in peach at 15° C. to 26 days for individuals reared in chicozapote at 30° and 32.5°. Development in peach was invariably longer than in chicozapote, the difference being in the feeding or larval stage.

When the rate of development in each fruit, measured by the reciprocal of time in days, was plotted against temperature, linear regression lines were produced.

STUDIES IN THE CHEMISTRY OF GRASS SILAGE[1]

By J. G. ARCHIBALD

Research professor of animal husbandry, Massachusetts Agricultural Experiment Station

INTRODUCTION

In connection with an attempt to find substitutes for molasses as preservatives for silage in our wartime economy, and to ascertain whether good quality grass silage could possibly be made without any preservative, a rather extensive study of the biochemistry of numerous kinds and types of silage was carried on during the seasons 1942–43 and 1943–44.

The literature of silage making is voluminous and no good purpose would be served by attempting to review all or even any considerable part of it here. The reader is referred to reviews by Bender and Bosshardt (*2*),[2] Hopkins and Ripley (*5*), LeClerc (*6*), and Woodward and Shepherd (*18*). Earlier chemical studies are confined pretty largely to routine fodder analyses which, while they furnish information on feeding value, shed little light on the fermentation process or on those end products (especially volatile acids and ammonia) which determine the quality and palatability of the silage. Noteworthy exceptions to this statement are the work of Dox and Neidig (*3*, *4*), Neidig (*9*), and Swanson and Tague (*15*), although Dox and Neidig worked only with the corn plant. Recent contributions on the subject which are of significance here include the work of Watson and Ferguson (*17*), Perkins (*11*, *12*), and Stone et al. (*14*).

EXPERIMENTAL METHODS AND MATERIAL

Forty samples of silage representing various crops and treatments were analyzed to determine moisture content, pH values, total nitrogen, water-soluble nitrogen, carotene, volatile bases, total acidity, amino acids, lactic acid, acetic acid (total and free), and butyric acid (total and free). In addition, the first five of the above entities were determined in nine samples of the fresh green crops before ensiling. The methods used were: For moisture, the Brown-Duvel toluene distillation procedure as modified by the Association of Official Agricultural Chemists (*1*, *p. 353*); pH values, a water extract of the finely chopped silage, using a Coleman pH meter with glass electrode; total nitrogen in the fresh sample by the usual Kjeldahl procedure; water-soluble nitrogen, the method of Watson and Ferguson (*17*); carotene, the method of Wall and Kelley (*16*); volatile bases, total bases, total

[1] Received for publication October 12, 1944. Contribution No. 538 of the Massachusetts Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 287.

acidity, and amino acids by the method of Foreman as outlined by Watson and Ferguson (*17*); acetic and butyric acids by the method of Wiegner as given by Watson and Ferguson (*17*); and lactic acid by difference as suggested also by these workers, i. e., total acidity—(amino acids+acetic acid+butyric acid)=lactic acid. In addition to the above the customary fodder analyses were also made.

In sampling from the large silos on the college farm small handfuls of silage were taken here and there over the entire area of a freshly exposed surface, sufficient to fill a friction-covered tin pail of 6-quart capacity. Samples submitted from farms were usually of much greater volume (a bushel or more), thus affording an opportunity for careful subsampling. Samples from the miniature silos were taken from all through that part of the mass of silage which was not obviously spoiled by rot or mold. All determinations except fat, fiber, and ash were made on the fresh samples finely chopped with scissors just before weighing. Total nitrogen was determined in both fresh and dry material.

The samples may be divided into 3 lots: (1) A series of 20 samples (16 of silage and 4 of fresh unensiled crops) which were obtained in a study of 2 crops treated with various preservatives in miniature silos, during the season of 1942–43; (2) a series of 19 samples of different origin and nature, obtained also in 1942–43, 13 of which were sent in by cooperating farmers [3] interested in the project, the other 6 being obtained from the large silos on the college farm; and (3) a series of 10 samples (5 of silage and 5 of the corresponding crops before ensiling) obtained from 1 of the large silos on the college farm during the season of 1943–44.

As already noted, the first lot of samples came from miniature silos. The objective of the study with these was to ascertain whether results might be obtained from small containers which would be similar to those secured from large silos. Statements in the literature are conflicting on this point; it is obvious that if small containers could be used with confidence in the results obtained, progress toward the main objectives of the investigation would be greatly accelerated.

The containers employed for the purpose were heavy glass cylinders, 9.5 inches inside diameter, and 39 inches high, open at both ends. The volume was slightly under 1.5 bushels. Sixteen such containers were used in accordance with the schedule in table 1. They were subjected to as much pressure as was practicable (120 pounds per square foot) and were kept in a darkened room.

The second lot of samples was obtained for the most part without resort to any special experimental set-up. All were from large silos (100 tons capacity or more) and represented conditions regularly existent in practical operations. Two of them can be compared with certain samples in the previous group; they were from the same lot of mixed grass ensiled at the same time, the only difference being that they were from 100-ton silos on the college farm. Thus a direct comparison was provided between large silo and small container on the same lot of grass and with the same preservative.

[3] Acknowledgment is made of the cooperation of these men and more especially, of the interest taken by Herbert A. Brown, county agricultural agent at Concord, Mass., who was instrumental in obtaining the samples.

TABLE 1.—*General outline and sample identification* [1]

LOT 1 (FROM MINIATURE SILOS)

Mixed grasses: [2]
1. The fresh, unensiled crop; Nos. 5, 6, 7, and 8 represent the ensiled product.
2. As in 1; Nos. 9, 10, 11, and 12 represent the ensiled product.

Alfalfa: [3]
3. Unwilted. The fresh crop; Nos. 13, 14, 15, 16, and 17 represent the ensiled product.
4. Wilted for 27 hours; Nos. 18, 19, and 20 represent the ensiled product.

Mixed grass silage:
5. No preservative.
6. Lactic acid culture—66 lbs. (*Lactobacillus bulgaricus*).
7. Salt—18 lbs.
8. Lactic acid culture and salt.
9. As in No. 5 plus a little water.
10. As in No. 6 plus a little water.
11. As in No. 7 plus a little water.
12. As in No. 8 plus a little water.

Alfalfa silage:
13. Unwilted. No preservative.
14. Unwilted. Lactic acid culture.
15. Unwilted. Molasses—90 lbs.
16. Unwilted. Salt.
17. Unwilted. Lactic acid culture and salt.
18. Wilted. No preservative.
19. Wilted. Lactic acid culture.
20. Wilted. Molasses.

LOT 2 (MISCELLANEOUS SAMPLES—ALL SILAGES)

Mixed grasses:
21. No preservative. Similar to 5 and 9, except from a large silo.
22. Lactic acid culture. Similar to 6 and 10, except from a large silo.
23. Lactic acid culture and salt—15 lbs.
24. Molasses—40 lbs.

Mixed grass and clover:
25. Lactic acid culture and salt—8 lbs.
26. Molasses—50 lbs.

Timothy and clover:
27. No preservative.
28. Molasses—amount not known.
29. Molasses—35 lbs.

Oats:
30. No preservative.
31. Lactic acid culture and salt (16 lbs.)

Oats and peas:
32. No preservative } From the same silo 7 weeks
33. Do. } apart.
34. Salt—10 lbs.

Winter wheat:
35. Salt—a pailful to a load.

Soybeans:
36. Molasses—amount not known.

Sudan grass, millet, and sorghum:
37. Molasses—amount not known.

Corn:
38. No preservative } Included for comparison.
39. Do. }

LOT 3 (FROM LARGE EXPERIMENTAL SILO)

Mixed grass and clover:
40. The freshly chopped crop after preservative has been added, but before ensiling.
41. Do.
42. Do.
43. Do.
44. Do.
45. Molasses (75 lbs.) and urea (10 lbs.). No. 40 after ensiling.
46. Urea (10 lbs.) No. 41 after ensiling.
47. Ground wheat—100 lbs.—No. 42 after ensiling.
48. Ground wheat—150 lbs.—No. 43 after ensiling.
49. Ground wheat—200 lbs.—No. 44 after ensiling.

[1] Amounts of preservative given are per ton of green crop; where not stated they are the same as previously noted in the lot in question. In the large silos lactic acid culture was applied at several levels, being first diluted with water; a sprinkling can full of the diluted culture was used at each level.
[2] Headed to some extent but not in bloom.
[3] In full bloom.

The third lot was from a definite experimental set-up in a 100-ton silo; five different lots of silage were treated as outlined in table 1. Assurance that each sample of silage would represent material identical, or nearly so, with that of the corresponding sample of fresh crop taken at filling time was provided for by the following procedure: A sample of the freshly chopped crop to which the specific preservative had been added in routine fashion was taken from the silo about midway of each lot; at the same time a clean burlap grain sack was filled with the same material as the sample, and, properly tagged, was buried in the center of the silo. From each sack when reached as the several lots were fed, the corresponding silage sample was secured. Table 1 outlines the scope of the work and serves to identify the several lots and samples.

CHEMICAL ANALYSES

Limitations of space preclude presentation here of the large accumulation of detailed data. Instead, general summaries and emphasis on high lights and on significant differences are depended upon to tell the story. It may be of interest to note in passing that the work involved over 800 chemical determinations (not counting parallels) in addition to several hundred other observations most of which were organoleptic. All values except moisture, pH, and carotene are reported as percentages in the dry substance; the first two exceptions are of course the values for the fresh silage; the carotene values are expressed as milligrams per pound of dry silage. Not all of the various entities determined are reported; it became evident upon critical examination that some of them showed only minor variations from sample to sample, and hence contributed nothing of significance to the findings. Table 2 summarizes the results from two standpoints—variation due to (1) kind of crop ensiled, and (2) kind of preservative used.

TABLE 2.—*Composition of silage as influenced by kind of crop and by preservative used* [1]

Crop ensiled and preservative used	pH	Volatile bases [2]	Total acidity [3]	Lactic acid	Acetic acid	Butyric acid	Carotene
Crop:		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Mg. per pound*
Alfalfa	4.5	0.76	14.41	4.75	5.49	0.66	46
Mixed grasses	5.2	1.49	11.43	.16	1.84	7.29	80
Mixed grasses and clover	4.6	.76	9.63	3.64	1.50	2.98	68
Timothy and clover	4.5	.78	12.13	3.26	3.52	2.80	77
Oats	5.2	.60	7.65	2.06	1.65	2.40	33
Oats and peas	4.5	.46	10.96	3.96	2.66	.50	39
Winter wheat	5.4	.81	11.28	None	2.26	6.83	117
Sudan grass, millet, and sorghum	4.5	.58	9.66	1.13	2.28	4.42	13
Soybeans	4.3	.54	13.00	4.73	4.42	1.64	28
Corn (included for comparison)	3.8	.26	14.64	7.58	5.22	None	27
Preservative:							
None [4]	4.8	.92	11.26	2.58	2.69	2.87	58
Lactic acid culture	4.7	1.14	12.89	2.62	3.27	4.21	63
Salt	5.0	1.08	11.61	1.04	2.88	5.13	77
Lactic acid culture and salt	5.3	1.14	11.23	.19	3.34	5.38	63
Molasses	4.5	.74	12.48	4.26	3.21	3.04	62
Molasses and urea	5.2	1.70	10.63	4.16	1.92	2.74	63
Urea	5.6	3.80	13.11	None	5.68	6.44	130
Ground wheat	4.3	.50	9.02	5.06	1.04	2.49	54

[1] The values in this table are composites, and include all samples listed under the specific corresponding category in table 1.

[2] Volatile bases are expressed as percentage of ammonia in the dry substance.

[3] Total acidity is expressed as the weighted percentage of amino, lactic, acetic, and butyric acids in the dry substance of each sample, based on the molecular weights of the acids in question. (Amino acids were calculated as an average of aspartic and glutamic acids, based on their molecular weights.)

[4] Does not include the values for corn silage.

Variations due to the crop used were more significant than those due to the action of preservatives. The principal points of interest are:

(1) In general the legume silages developed a higher acidity than those made from grasses or small cereals; also the presence of a legume in a mixture resulted in higher acidity. The inverse relationship held true for volatile bases, which is what would be expected.

(2) For the most part also the legumes developed a higher total acidity. Differences, however, are more evident in the type of acidity than in the total amount. The legume silages were characterized by a relatively high content of lactic and acetic acid and their influence

in this respect is also evident in the mixed crop silages; the grass silages and certain of the small cereals, on the other hand, had a relatively high butyric acid content and little or no lactic acid.

(3) The two lots of corn silage offer a striking contrast to the grass silages in their low pH, values very low volatile base content, high lactic and acetic acid, and absence of butyric acid. This seems an appropriate point at which to make the general observation that the most strongly acid-smelling silages have frequently been the least acid, as judged either by pH or by total acidity as determined by titration. This may seem paradoxical until one considers that the type of fermentation which develops butyric acid is likely also to develop more ammonia and other bases which neutralize a portion of the acid and keep the pH relatively high and the titration values low. The better quality silages with mild, pleasant odor were almost invariably more acid than those which sense of smell alone would rank as much more acid. The acid in the better quality silages was largely lactic, which is nonvolatile and of relatively mild odor, and their volatile base content was usually quite low.

(4) Of the preservatives used molasses and ground wheat seem to have been the only ones that were at all effective. They kept the break-down of protein at a relatively low level, as evidenced by low content of volatile bases; favored the formation of lactic acid; and limited the formation of butyric acid. Salt and lactic acid cultures were in many respects worse than no preservative at all; they encouraged the formation of volatile bases and butyric acid and inhibited the development of lactic acid, some of the samples showing none at all. Urea was evidently entirely unsuited to the purpose; the idea in using it was to increase the nitrogen content of the silage and hence the potential protein level for ruminants. It seems apparent from the high pH and the very high content of volatile bases in this lot of silage that a considerable portion of the urea must have been converted into ammonia. That some of it was lost by leaching is evident from the increased nitrogen content of the leachings from this particular silo. The average figure for 4 years previously was 0.30 percent nitrogen; when urea was used it rose to 0.56 percent. The silage made with urea alone as a preservative had a very objectionable odor and was unpalatable to milking cows. The silage made by using ground wheat as a preservative had a mild, pleasing odor and was very palatable. The herdsman in charge observed that it was the best quality grass silage he had fed in our 8 years of experience. In table 3 is presented a summary of the proximate analyses,[4] together with some interesting contrasts between certain of the silages and the fresh crops from which they originated.

Changes in composition due to the silage fermentation show a definite trend regardless of the nature of the crop ensiled. Relatively the greatest losses are in carotene and the greatest gains are in ether extract. The gains in ash and fiber doubtless are apparent only, because of losses of a similar magnitude in protein and nitrogen-free extract. The gain in ether extract is actual, since the organic acids formed by fermentation from the carbohydrates are ether-soluble,

[4] Acknowledgment is made of the services of Albert F. Spelman, assistant chemist, and Leo V. Crowley and C. Tyson Smith, junior chemists, in the Feed Control Laboratory of this station, who made the fodder analyses under the direction of John W. Kuzmeski, senior chemist, and Philip H. Smith, chief chemist.

whereas carbohydrates are not soluble in anhydrous ether. High carotene values did not necessarily occur in silages that were of good quality in other respects; e. g., note the very high value for the winter wheat silage. In most other respects this was about the poorest quality silage examined; a similar condition was noted in a lot of mixed grass and clover preserved with urea (see table 2). Stage of maturity of the crop probably had a good deal to do with this. In the case of the silage preserved with urea it may be that the type of fermentation induced by the high pH altered certain noncarotene pigments sufficiently to permit their estimation as carotene.

TABLE 3.—*Average proximate composition of silages from various crops and crop combinations, and of some fresh, unensiled crops from which certain of the silages were prepared* [1]

Kind of crop	Moisture as sampled or received	pH	Carotene	Percentages in the dry matter				
				Total ash	Crude protein	Crude fiber	N-free extract	Ether extract
	Percent		*Mg. per pound*					
Alfalfa	62.3	6.0	133	7.3	17.8	35.7	37.4	1.8
Alfalfa silage	68.2	4.5	46	9.3	16.8	38.5	32.8	2.6
Grasses, mixed	75.0	6.1	157	7.3	12.8	33.9	43.3	2.7
Grasses, mixed, silage	74.8	5.2	80	[2] 10.2	10.0	38.7	38.0	3.1
Grasses and clover	68.1	6.2	58	8.0	12.1	32.8	44.9	2.2
Grass and clover silage	68.9	4.6	68	7.8	10.5	37.2	41.0	3.5
Timothy and clover silage	73.8	4.5	77	10.6	12.9	34.7	38.8	3.0
Oat silage	70.6	5.2	33	9.2	9.1	38.0	40.3	3.4
Oat and pea silage	67.3	4.5	39	8.7	10.4	35.8	41.7	3.4
Winter wheat silage	73.9	5.4	117	12.6	8.0	41.4	34.5	3.5
Sudan grass, millet, and sorghum silage	79.7	4.5	13	7.9	4.6	44.5	40.9	2.1
Soybean silage	71.0	4.3	28	19.7	10.9	30.4	37.2	1.8
Corn silage (for comparison)	74.2	3.8	27	6.0	8.4	35.3	47.6	2.7

[1] The values in this table are composites, and include all samples listed under the specific corresponding category in table 1.
[2] This figure is due in part to the use of salt in some of the silages.

Table 4 shows the composition of material ensiled in the miniature silos referred to earlier in comparison with material from the same lot of grass ensiled with the same treatment in two 100-ton silos. Except for carotene the differences are rather small; what there are indicate that fermentation took place on a more extensive scale in the large silos. The differences in carotene content are arresting; if typical of what may occur in large silos they represent a need for further investigation in order that such excessive losses of this important nutrient may be prevented. These losses are on a par with those occurring in dry hay. It should be mentioned that the small containers did not prove satisfactory. Spoilage losses were high in most of the containers, averaging over 18 percent, due chiefly to rotting of the upper layers. In the containers in which wilted alfalfa was stored the losses were excessive, averaging over 60 percent, due to penetration of mold. Fermentation losses were normal in most cases, averaging about 9 percent with some under 5 percent. Evidently conditions existent in a large silo are not readily duplicated on a small scale.

TABLE 4.—*Composition of silage from the same lot of grass ensiled in small silos (1.5 bushel capacity) and large silos (100 tons capacity)*

Type of silo and treatment of silage	Moisture when sampled	pH	Carotene	Percentages in the dry matter									
				Volatile base	Total acidity	Lactic acid	Acetic acid	Butyric acid	Total ash	Crude protein	Crude fiber	N-free extract	Ether extract
	Pct.		*Mg. per pound*										
Small silos—no preservative	78.3	5.1	107	1.74	12.15	None	1.92	7.89	7.6	10.2	38.0	40.6	3.6
Large silos—no preservative	73.4	5.4	25	1.59	13.22	None	.80	8.08	8.7	9.2	40.4	38.2	3.5
Small silos—lactic acid culture	76.4	5.0	84	1.44	10.91	None	2.15	7.55	8.9	10.3	38.8	39.0	3.0
Large silos—lactic acid culture	71.4	4.9	32	1.36	12.61	0.73	2.46	5.70	9.8	9.3	39.8	37.9	3.3

In table 5 appear the contrasts in composition due to wilting of alfalfa. Changes in composition before ensiling caused by wilting seem to have been confined to a material reduction in carotene content and to rather minor and probably only relative increases in protein and nitrogen-free extract with a corresponding reduction in fiber. Changes after ensiling are of much more significance; note especially the lactic acid-acetic acid relationship in the wilted and unwilted silage, and the much lower fiber and higher protein and nitrogen-free extract of the wilted silage. Insofar as these silages are concerned wilting resulted in a very different type of fermentation and final product. It may be that the influence of mold enters in here; the excessive development of mold in the wilted silages has been mentioned already, but it should be noted that care was taken to discard all visible signs of mold when the samples were taken for analysis.

CHLORIDE CONTENT OF SILAGE

In the course of the work the question arose as to whether the practice of using salt as a silage preservative might not at times introduce an excessive amount into the rations of livestock, and this in turn suggested the need to know how much salt (or total chlorides) occurs in silage naturally. A search of the literature showed very little information on this point, so a determination of the chloride content of these 40 silages was made a part of the routine procedure. The determination was made on suitably diluted duplicate aliquots of the water extract by titration with N/10 silver nitrate. A 10-percent solution of potassium chromate was used as indicator, the end point being determined by the first permanent color change visible against the background of a glazed white porcelain evaporating dish. The results are summarized in table 6.

The chloride values were highest in the silages made from small grains and lowest in those made from legumes. Salt increased the chloride content to a value about 2½ times that found in the silages with which no preservative was used. Other preservatives were without substantial effect. Consideration of the effect of the added salt upon feeding practice and animal health is appropriate here.

TABLE 5.—*Composition of alfalfa, wilted and unwilted, and of the corresponding lots of silage made from it*

Alfalfa	Moisture when sampled	pH	Carotene	Percentages in the dry matter									
				Volatile bases	Total acidity	Lactic acid	Acetic acid	Butyric acid	Total ash	Crude protein	Crude fiber	N-free extract	Ether extract
	Per cent		*Mg. per pound*										
Unwilted—before ensiling	73.5	6.0	156	(1)	(1)	(1)	(1)	(1)	7.4	17.1	37.5	36.3	1.8
Wilted—before ensiling	49.0	6.0	110	(1)	(1)	(1)	(1)	(1)	7.2	18.4	34.0	38.7	1.7
Unwilted—after ensiling	77.7	4.6	49	0.98	15.58	2.68	8.15	0.66	9.9	15.7	41.6	30.0	2.8
Wilted—after ensiling	52.5	4.2	40	.38	12.47	8.20	1.05	None	8.2	18.6	33.4	37.6	2.2

[1] Not determined.

TABLE 6.—*Average chloride content of different kinds of silage*

Crop [1]	Chlorides in the dry matter [2]	Preservative	Chlorides in the dry matter [2]
	Percent		*Percent*
Alfalfa	0.80	None	1.10
Mixed grasses	1.13	Lactic acid culture	1.03
Mixed grasses and clover	1.06	Salt	2.77
Timothy and clover	1.26	Lactic acid culture and salt	2.46
Oats	1.56	Molasses	1.09
Oats and peas	1.19	Molasses and urea	1.17
Sudan grass, millet, and sorghum	1.43	Ground wheat	.88
Soybeans	.21		
Corn	.54		

[1] All samples in which salt was used as a preservative have been excluded from the average values for the several crops.

[2] To convert these values to terms of NaCl, multiply by 1.65; to convert them to an average basis of fresh silage as fed, multiply by 0.3.

A little calculation shows that from the silages preserved with salt a cow consuming 40 pounds of fresh silage daily would get 0.55 pound of salt daily from this source alone. Assuming that she also consumes 10 pounds of a grain mixture with its usual salt content of 1 percent, she would get from these two sources somewhat over 10 ounces of salt daily; there is also the unknown quantity contained in whatever other roughage she might consume (a cow eating 40 pounds of silage usually eats 10 to 12 pounds of dry hay in addition). Information in the literature as to the amount of salt a cow requires is very meager; Maynard (*7, p. 435*) makes the statement that "One to two ounces of salt daily should be adequate for milking cows." Although undoubtedly cows can tolerate somewhat larger amounts than this, it is extremely doubtful whether they should receive for any considerable length of time as large an amount as 10 ounces daily. Furthermore, this is based on only the average amount found in the samples to which salt had been added; the maximum amount of chlorides found was 4.14 percent in the dry matter. Figured on the same basis as above a cow's daily salt intake on this particular lot of silage would be approximately 14 ounces; undoubtedly this is excessive.

EVALUATION OF CERTAIN METHODS

In the course of the investigation there were opportunities to compare certain analytical procedures, notably those dealing with the determination of total nitrogen and with carotene.

It has been recognized for years that in general considerable losses of nitrogen occur in all types of biological material if it is dried and ground, as for routine fodder analyses, before determining total nitrogen. Yet one finds in the literature instances of recent work where this fact has been ignored. For comparative purposes total nitrogen was determined in the fresh material immediately upon its receipt at the laboratory and again after drying and grinding. Carotene was determined by the phasic separation procedure outlined in the Official Methods (*1*) and by the chromatographic method described by Wall and Kelley (*16*).[5] The results are shown in table 7.

Considering nitrogen first, the interesting thing is the great variation in the error due to determination after drying. On the whole, it was much larger in the silages than in the unensiled crops, but the average errors for some are so small that they might almost be classed as experimental error; e. g., that for corn or for mixed grass and clover. One might be tempted to place in this class the positive error for silage preserved with ground wheat, except for the fact that it was consistent and the average represents three different lots of silage. Apparently the ground grain served to fix volatile nitrogenous bases and thus to prevent the customary loss on drying. Molasses seems to have had a similar though less marked effect.

TABLE 7.—*Comparison of (a) nitrogen determination, and (b) carotene determination in green crops and in silages by 2 methods*

Material	Percentage of nitrogen in the dry matter		Percentage loss due to drying	Milligrams of carotene per pound of dry matter		Result in (2) is lower by (percent)—
	Determined in the fresh material	Determined in the dried and ground sample		(1) By phasic separation	(2) By chromatography	
Crops before ensiling:						
Alfalfa	3.05	2.85	5.6	[1]	[1]	[1]
Mixed grasses	2.08	2.05	1.4	[1]	[1]	[1]
Mixed grasses and clover [2]	1.91	1.80	5.8	63	58	7.9
Kinds of ensilage:						
Crop:						
Alfalfa	3.10	2.69	13.2	44	36	18.2
Mixed grasses	2.29	1.58	31.0	113	84	25.7
Mixed grasses and clover [2]	1.80	1.76	2.2	103	83	19.4
Timothy and clover	2.25	2.07	8.0	138	93	32.6
Oats	1.76	1.45	17.6	32	30	6.2
Oats and peas	1.77	1.67	5.6	39	19	51.3
Winter wheat	1.97	1.27	35.5	[1]	[1]	[1]
Sudan grass, millet, and sorghum	1.04	.74	28.9	29	13	55.2
Soybeans	2.04	1.75	14.2	56	28	50.0
Corn	1.37	1.35	1.5	46	27	41.3
Preservative:						
None	2.15	1.87	13.0	71	51	28.2
Lactic acid culture	2.63	2.10	20.1	90	59	34.4
Salt	2.43	1.63	32.9	84	67	20.2
Lactic acid culture and salt	2.11	1.52	28.0	89	67	24.7
Molasses	2.37	2.14	9.7	99	70	29.3
Molasses and urea	2.21	1.56	29.4	62	47	24.2
Urea	2.99	1.45	51.5	139	130	6.5
Ground wheat	1.84	1.88	+2.2	68	51	25.0

[1] Comparative figures not available.
[2] For obvious reasons values for those samples of mixed grass and clover to which urea has been added are not included in the averages.

[5] Material assistance rendered and valuable suggestions made by John W. Kuzmeski, senior chemist in the Feed Control Laboratory of this station, are gratefully acknowledged.

Several investigators (*8*, *13*, *16*) have shown that when carotene is determined by alkaline extraction followed by phasic separation as outlined in the official method, varying amounts of noncarotene chromogens remain in the carotene fraction and result in carotene values that are too high. Moore (*8*) in particular has shown that this error is much more significant in silages than in fresh green crops; the results in table 7 confirm and extend his observations. In general, phasic separation gave carotene values about one-third higher than those obtained by chromatography, although the range was wide (from 6 percent to 55 percent higher). Variation was greatest between crops; except for the rather small difference when urea was used, variability in this respect between silages treated with different preservatives was slight, the differences between the two methods being of the same general order (about 25 percent), regardless of preservative used.

SUMMARY

A biochemical study has been made of 40 samples of different kinds of silage stored with various preservatives and of 9 samples of fresh green crops before ensiling which corresponded in identity with certain of the silages.

Variations due to the kind of crop used were more significant than those due to the action of the preservative.

In general legume silages had lower pH values and higher total acidity than silages made from grasses or small grains. The type of acidity also was different; legume silages were characterized by a high lactic acid content while the others showed relatively large amounts of acetic and butyric acids.

The most strongly acid-smelling silages were frequently the least acid, as judged by either pH or total titratable acidity. An explanation of this apparent paradox is offered.

Corn silage offered a striking contrast in composition to grass silage.

Of the preservatives used, molasses and ground wheat seem to have been the only ones at all effective. Salt and lactic acid cultures were in many respects inferior to no preservative at all. Urea was apparently entirely unsuitable for the purpose.

Changes in proximate composition due to the fermentation process showed a definite trend regardless of the nature of the crop ensiled. High carotene values did not necessarily characterize silages that were of good quality in other respects. Carotene losses in some of the large silos were excessive.

Small containers utilized in an effort to speed up the work did not prove satisfactory, losses due to rotting and mold being excessive. Evidently conditions existent in a large silo are not readily duplicated on a small scale.

Silage made from wilted alfalfa was quite different in composition from that made from the unwilted crop.

The chloride content of some of the silages in which salt was used as a preservative was above the optimum for reasonable intake of salt, and in some cases it was excessive.

Losses in total nitrogen, as indicated by determining this element in both the fresh and the dried, ground samples, were very variable, being much higher in the silages than in the corresponding green crops.

Certain preservatives (molasses and ground wheat) tended to minimize this loss.

The chromatographic procedure for determining carotene gave results consistently somewhat lower than those obtained by the official phasic separation procedure.

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THE RELATION BETWEEN NUMBER OF EARS OPENED AND THE AMOUNT OF GRAIN TAKEN BY REDWINGS IN CORNFIELDS [1]

By D. W. HAYNE [2]

Research assistant zoologist, Michigan Agricultural Experiment Station

INTRODUCTION

Damage to corn (*Zea mays* L.) by flocks of the eastern redwing (*Agelaius phoeniceus phoeniceus* (L.)) occurs in local areas over a large part of the United States. The pattern of damage in a cornfield at any particular moment is the expression of certain feeding habits of birds in flocks. This paper presents the results of an attempt to describe the progressive increase of damage in algebraic terms and to test the validity of the equations developed by application to data gathered in damaged fields. The work was undertaken to obtain a better understanding of the biology of redwing feeding in cornfields as part of an experimental testing of bird-scaring devices. The tests were made in collaboration with H. A. Cardinell, during four summers, 1939–42.[3] It is assumed that these records of terminal damage in a number of fields are equivalent to records at successive stages of damage in a single field, at least as far as the relation between number of ears opened and amount of grain taken is concerned.

The diet of redwings is composed largely of weed seeds and insects. However, when the developing grain of the corn is in the soft or milk stage, the birds may attack the fields and continue their raids until the grain has become hard and ripe. The actual damage caused by the birds consists in ripping open the husks of the ears and removing some of the grain. The amount of grain taken from an ear by one bird at one visit is small, usually less than 5 percent of the grain in the ear. When feeding, the redwings flutter from cornstalk to cornstalk, stopping to feed at an ear for only a short time. Redwings feed in flocks of up to several hundred birds, but within a feeding flock relatively few birds seem to be actually eating at any one moment. Often an undisturbed feeding flock will pass slowly across a cornfield, the individual birds stopping to feed for a few moments while the flock as a whole progresses. A feeding flock often remains a moderately compact unit, and at alarm the birds may rise into the air together. Redwings appear to be easily disturbed, and often a flock will rise from one cornfield and fly to feed in another. After a short time the same flock, or another one, may be back in the first field, feeding again. Thus, a single bird does a small amount of feeding on each of a large number of ears, apparently spread over a considerable area of the countryside. Consequently, the total damage in any one field is the sum of a large number of small amounts of

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[3] CARDINELL, H. A., and HAYNE, D. W. CORN INJURY BY RED WINGS IN MICHIGAN. Mich. Agr. Expt. Sta. Tech. Bul. [In press.]

grain eaten during a period of several weeks while the grain is ripening.

Redwings show a distinct preference for certain classes of ears of corn. In any field, the first ears to be damaged are those that first reach the milk stage. The ears of greater length and diameter, that is, the better ears, are apparently preferred. There may well be other criteria for preference.

DERIVATION OF EQUATIONS

The existence of preferred classes of ears within a field will be ignored for the moment, to be brought into the picture later. A uniform field is assumed first, where every ear has the same chance of being selected.

If n feeding birds are in a field containing N ears, and each bird damages one ear, the proportion of the ears damaged (x) will be

$$x=\frac{n}{N}$$

and the proportion not damaged (x') will be

$$x'=1-\frac{n}{N}$$

If selection is at random, the proportion not damaged after v visits will be

$$x'=\left(1-\frac{n}{N}\right)^v$$

from which

$$v=\frac{\log x'}{\log\left(1-\frac{n}{N}\right)} \tag{1}$$

If each bird takes b (for "bite") ears at each visit (as explained previously, b is probably small, much less than 1), then the flock will take nb ears at each visit. In v visits the flock will take a certain proportion of the grain in the field, represented by y in the equation

$$y=\frac{nbv}{N} \tag{2}$$

Substituting the value of v from equation (1) in equation (2)

$$y=\frac{nb\log x'}{N\log\left(1-\frac{n}{N}\right)}$$

which may be stated in the form

$$y=\frac{b\log x'}{\log\left(1-\frac{n}{N}\right)^{\frac{N}{n}}} \tag{3}$$

The denominator of (3) approaches a limit when N is many times as large as n, for the quantity

$$\left(1-\frac{n}{N}\right)^{\frac{N}{n}} \tag{4}$$

when expanded takes the form

$$1-\frac{\left(\frac{n}{N}\frac{N}{n}\right)+\frac{N}{n}\left(\frac{N}{n}-1\right)\left(\frac{n}{N}\right)^2}{2}-\frac{\frac{N}{n}\left(\frac{N}{n}-1\right)\left(\frac{N}{n}-2\right)\left(\frac{n}{N}\right)^3}{2\cdot 3}+$$

$$\frac{\frac{N}{n}\left(\frac{N}{n}-1\right)\left(\frac{N}{n}-2\right)\left(\frac{N}{n}-3\right)\left(\frac{n}{N}\right)^4}{2\cdot 3\cdot 4}-\text{etc.}$$

The value of the sum of the terms of this expansion is approximately 0.3660 when $\frac{n}{N}$ is 0.01; 0.3670 when $\frac{n}{N}$ is 0.005; and 0.3679 when $\frac{n}{N}$ is so small as to have no effect on the value. As $\frac{n}{N}$ approaches zero, equation (4) approaches the value of e^{-1}.

In field observations flocks of birds larger than several hundred individuals were rarely observed feeding in cornfields, although larger flocks might often be seen at the roosting areas. The number of ears in a single acre of corn may be estimated as 10,000 or more. Therefore the quantity (4) may safely be used as a constant, of magnitude about 0.367. Substituting in equation (3)

$$y=\frac{b\log x'}{\log .367}$$

which reduces to

$$y=-2.30b\log x' \tag{5}$$

Since X' is the proportion of ears not damaged, equation (5) is more useful in the form

$$y=-2.30b\log(1-x)$$

or

$$y=k\log(1-x) \tag{6}$$

where x is the proportion of ears damaged and k is $-2.30b$.

Thus far we have assumed that selection of ears in a field is at random. However, as previously stated, this is not true over a whole field. Therefore let equation (6) refer only to one class within which there exists uniform probability of selection. One such class might be thought of as containing all ears about 6 inches long, of about the same diameter, about the same stage of maturity, and uniform in other respects. There will be a number, c, of these classes in a field, each class distributed over the whole field, extending side by side with all the other classes which are spread over the same area. Any one class will have a relatively small number of ears, such as N_1, and the sum of all the ears in all the classes will, of course, be the total

number of ears in the field, or N. The various classes will not necessarily contain an equal number of ears.

The fact that the number of ears in any one class may approach the total number of birds in a flock brings up the question whether the fraction $\frac{n_1}{N_1}$, pertaining to class 1, is still small enough to make equation (4) a constant. Since any one class of ears may be widely scattered over the whole field, since the feeding flock is concentrated in only a part of the field, since only part of the birds in a flock seem to feed at any moment, and since the feeding birds are attacking at one time a number of classes of ears, it is probable that n_1, the effective number of birds attacking class 1, will be quite small. Thus the conditions under which (4) is approximately constant will be maintained, and the course of damage within each class may be described by equation (6), as applied to class 1,

$$y_1 = k \log (1 - x_1)$$

The quantity k will be the same throughout all classes.

The actual amount of grain taken from class 1 will be

$$N_1 k \log (1 - x_1)$$

and the total amount of grain taken from the whole field will be the sum of as many such terms as there are classes. Dividing the total amount of grain taken by the total available ears, N, gives y, the proportion of the grain of a field taken by the birds; that is,

$$y = \frac{N_1 k \log (1 - x_1) + N_2 k \log (1 - x_2) + \ldots N_c k \log (1 - x_c)}{N}$$

or

$$y = k \left[\frac{N_1 \log (1 - x_1) + N_2 \log (1 - x_2) + \ldots N_c \log (1 - x_c)}{N} \right]$$

The right-hand side of this last equation is k times the logarithm of the weighted geometric mean of the proportion of the various classes of ears not damaged. Such a weighted geometric mean is not available. However, the figure at hand, the weighted arithmetic mean, may serve as an approximation of the geometric mean, although known to be somewhat larger.[4] In the one case where data were available, in a sample of 1,088 ears, the weighted geometric mean of proportion of ears damaged in 60 size classes was 0.896, while the weighted arithmetic mean of the same classes was 0.907. Thus the form of the generalized expression remains the same as (6), although now assumed to refer approximately to a whole field instead of a single class within the field.

APPLICATION OF METHOD TO THE FIELD RECORDS

The field records consist of 198 estimates of damage made in fields during the seasons of 1941 and 1942. In each field an estimate was made by classifying about 200 ears into 5 groups according to damage and then computing an average of the damage per ear. The percent-

[4] SIMPSON, G. G., and ROE, A. QUANTITATIVE ZOOLOGY. 414 pp., illus. New York and London. 1939. (See pp. 102, 146.)

age of ears opened was computed from the same field record. These 198 estimates have been reduced to 63 items by arranging them in groups according to the proportion of the ears opened and using the mean of each group as a single item. Each group contains the records of all fields falling within a class of 0.01 of the ears opened. For example, there are 4 fields having between 0.195 and 0.205 of the ears opened (20 percent of the ears), and these 4 fields average 0.034 of the grain removed (3.4 percent). The number of field records in the different classes varies from 26 estimates in the class of 0.01 of the ears opened to single records in a number of cases. In general, the less the damage the more numerous the field records.

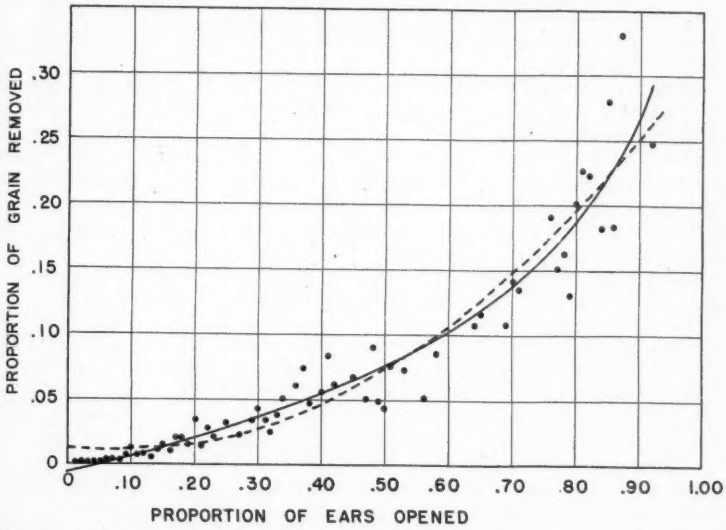

FIGURE 1.—Proportion of ears opened and proportion of total corn crop removed by redwings, based on 192 estimates of damage. Each dot represents the average of all fields having the same proportion of ears opened. The solid line is the curve derived from equation (7), $y=0.0062-0.2745 \log (1-x)$, and the broken line is derived from the equation $y=-0.0132-0.0621x+0.3633x^2$.

This combining of the data into classes is reflected in figure 1 in an apparent difference in variability at different levels of damage. This difference is, at least in part, an illusion caused by the averaging of more values for certain points than for others. This is not an important difficulty in this study, however, since the variability of the data is not of primary interest.

The 63 items (group means) were fitted to the derived curve (6), using logarithms and the method of least squares as ordinarily applied to fitting a straight line.[5] The equation of the curve derived is

$$y=-0.0062-0.2745 \log (1-x) \qquad (7)$$

[5] RICHARDSON, C. H. AN INTRODUCTION TO STATISTICAL ANALYSIS. Enl., 312 pp., illus. New York. 1935. (See p. 177.)

The intercept of this curve on the y axis is not located at zero where it should be,[6] but at -0.0062 (fig. 1). Consequently, use of the curve for translating values of proportion of ears opened to their theoretical equivalents in proportion of grain taken does not give satisfactory estimates for the range of from 0.01 to about 0.07 of the ears opened. The absolute deviations of the curve from the observed data are small, but the curve gives small negative values for the lower part of this range, a result which is biologically impossible.

The data in the range of 0.01 to 0.07 of the ears opened were fitted to the derived curve (6) independently, giving the somewhat different equation of

$$y=0.0005-0.1081 \log (1-x) \tag{8}$$

In this case the departure of the intercept on the y axis from zero is only 0.0005.

In the curve (7) fitted to the entire set of 63 items, a value of -0.2745 for k means a value of 0.119 ears for b (or about 12 percent of an ear per bird visit), since $k=-2.30b$. There are no field observations at hand to indicate whether this value is reasonable for the upper range of damage. It is known that 12 percent would be too high a value for the extreme lower end of the curve, for in field notes the first damage recorded on any ear resulting from the bird's visit when the ear is first opened was almost always less than 5 percent. However, the curve fitted to the whole set of data is in obvious disagreement with the data from the extreme lower part of the range, and the curve fitted to this lower range gives a value of 0.1081 for k with a corresponding value of 0.047 for b. This value of b is in much closer agreement with the field notes. There is thus a suggestion from these data that the birds may take a smaller amount of grain from ears that are just being opened than they do later, after damage has been started.

A parabola has been fitted to the 63 items by using the normal equations and the method of least squares.[7] This curve is shown, along with the theoretically derived curve (7), in figure 1. The equation of the parabola is

$$y=0.0132-0.0621x+0.3633x^2.$$

For purposes of comparison a straight line has been fitted to the data. This line is not shown in figure 1. The equation of the straight line is

$$y=-0.0317+0.2621x.$$

Using the method outlined by Rider, a comparison was made of the closeness of fit to the data of the line, the parabola, and the derived curve (7).[8] This method of comparison shows that either curve is better than the straight line, with a high degree of significance attached to the difference. Comparison of the parabola with the theoretically derived curve shows that although the theoretical curve fits the data more closely than does the parabola, the difference is not significant.

[6] When no ears have been opened no damage can have been done; negative damage is impossible.

[7] See footnote 5, p. —. (See p. 192.)

[8] RIDER, P. R. AN INTRODUCTION TO MODERN STATISTICAL METHODS. 220 pp., illus. New York. 1939. See pp. 120 and 124.)

Reasoning on biological grounds, however, it is felt that the theoretically derived curve has points of advantage over the parabola. The intercept of the parabola is at 0.0132 on the y axis. The minimum value of y is not at zero on the X axis, where it should be biologically, but at about 0.09, where the predicted value of y is 0.0106. The greatest deviation, however, from the facts as observed in fields is found in the parabola predicting a value of 0.314 of the grain taken when all the ears have been opened. Although none of the field records shows that more than 0.92 of the ears were opened, the trend of the data at this point does not suggest that the birds opened all of the ears in any field with as little as 0.314 of the grain taken. It is obviously dangerous to argue beyond the limits of the data at hand, but the field observations appear to support the derived curve against the parabola.

Field notes for the seasons 1939 and 1940 were recorded only in terms of the proportion of ears opened. The derived curve, as stated in equation (7), has been used in translating these records to the approximate equivalent in proportion of grain removed.[9] The results of this conversion of units seem satisfactory.

In this paper is presented a study of the relation between the proportion of ears of corn opened and the proportion of grain taken by redwings. The relationship was found to be fairly close, though not linear. Hence, the greater the number of ears opened the greater the loss of grain. There appears to be no necessity for assuming that birds return to an ear which has once been opened, merely because the ear has been opened.

Field observations and theoretical considerations support the belief that redwing selection of ears of corn within a cornfield is at random within certain preference classes. The field data conform to a curve derived upon these assumptions as closely as may be expected, in view of the limitations of the method used for measuring damage.

[9] See footnote 3, p. 289.

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